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United States
Department of
Agriculture

Forest Service

Pacific Northwest
Forest and Range
Experiment Station

Research Note
PNW-376
March 1981

Selection and Propagation of Highly Graft-Compatible Douglas-fir Rootstocks-- A Case History

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Abstract

Two populations of Douglas-fir trees were screened for graft compatibility. Two-stage testing procedures were used with either high or low intensity screening in the first step. Of 303 trees, 16 (5 percent) were found to be 90 to 100 percent graft compatible and suitable for seed orchard use as rootstocks. High intensity screening in the first stage was more effective than the low intensity screening in detecting highly compatible trees. Trees proven to be 90 to 100 percent compatible after second stage screening were propagated as rooted cuttings and used as parents in controlled pollinations to produce highly compatible seedling families. Approximately 120,000 cuttings have been gathered from 16 ortets and 212 field-planted ramets during the past 7 years. The future supply of cuttings and control-pollinated seed from these desired trees should be adequate to meet the rootstock requirements of most West Coast tree improvement organizations.

Keywords: Grafting, rootstocks, Douglas-fir, Pseudotsuga menziesii.

Introduction

Effective methods for detecting incompatible Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) grafts have been known and used for a number of years (Copes 1967, 1969, 1970, 1978). With these anatomical, phenotypic, and biochemical techniques, it is possible to detect incompatible stock-scion combinations and rogue them from seed orchards at an early age. Replacing incompatible grafts raises costs and delays seed production.

Some orchardists have reduced the problem by grafting troublesome scion clones on cuttings of rootstocks which have been anatomically tested and found compatible with the problem clones. This procedure usually requires testing each clone with a different rootstock. A more favorable approach would be to find trees which are highly compatible with most scion genotypes. Such a survey has not been made, and lack of widely compatible rootstocks has hindered the general use of clonal rootstocks.

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Another promising method of easing the graft-incompatibility problem is to use as seedling rootstock families derived from breeding graft-compatible parents. Studies on inheritance of graft rejection in Douglas-fir indicate primarily additive gene control (Copes 1973). A heritability value of 0.81 suggests that controlled pollinations between highly graft-compatible parents should result in compatible seedling progeny (Copes 1974). Unfortunately, suitable parents have not been identified, so breeding of each tree to produce rootstock has not occurred.

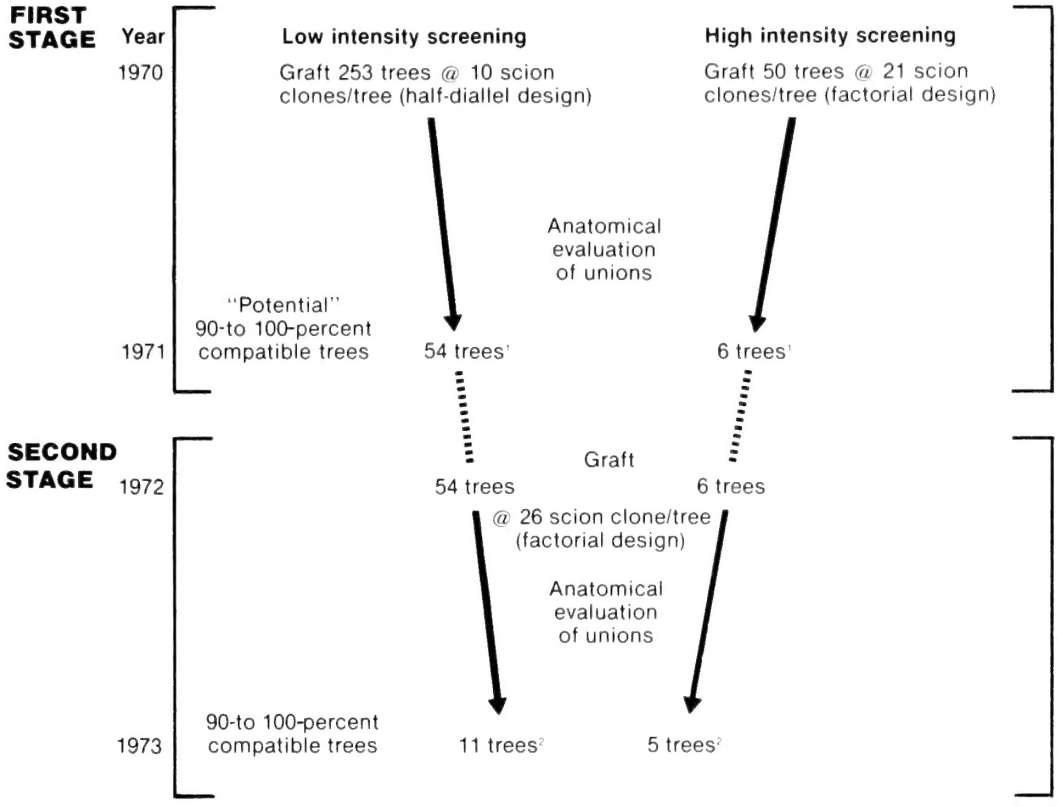
The following report describes a search for trees which are highly compatible and can be used to provide branch tips for cuttings or seed and pollen to produce compatible seedling rootstocks. In this study, trees which were 90 to 100 percent graft compatible were identified from populations of 50 and 253 trees. A two-stage method for testing graft-compatibility was used, with both high and low intensity screening as the first stage. Trees found potentially 90 to 100 percent compatible in the first stage were subjected to even more stringent testing in the second-stage.

Methods

Two juvenile Douglas-fir populations were sampled in 1970. One sample was 253 six-year-old trees at the Beaver Creek Seed Orchard, located 15 miles southwest of Corvallis, Oregon. The other sample consisted of fifty 9-year-old trees growing in a clone bank 6 miles north of Corvallis. Nothing was known about the compatibility of either population. Both samples grew in similar environments and received identical cultural treatments throughout the test. The two populations were selected because the trees were young enough to provide rootable cuttings, yet would soon be old enough to produce seed.

The first grafting was done in 1970. Two testing strategies were used in the first stage. The 253-tree population was given the low intensity test. It was divided into 23 sets of 11 trees (fig. 1). The grafting design was similar to a half-diallel mating design; each tree was used either as a scion or a rootstock with the other 10 trees of that set (fig. 2). Previous reciprocal grafting work had shown that it makes no difference in compatibility whether Douglas-fir clones are used as scions or stocks (Copes 1974). A total of 1,265 grafts was made. The high intensity test on the 50-tree sample involved grafting 21 scion clones on all 50 trees, making a total of 1,050 grafts. (The grafting design was similar to a 21-tester mating design) (fig. 1).

Figure 1.--Flow chart of screening methods.



[1] = Actual number of potential 90 to 100-percent-compatible trees after first stage testing.
[2] = Actual number of true 90 to 100-percent-compatible trees detected after second stage testing.

Graft unions from all first-stage tests were sacrificed at 18 months, microtomed into 25-µm cross sections, stained in safranin-O and fast green, and examined at 10 - 100X using a light microscope (Gnose and Copes 1975). Incompatible grafts were detected by the presence of wound-xylem areas in union zones at the start of the 2d year of growth (Copes 1970). Average compatibility was calculated for all 303 trees in the first-stage tests.

In 1972, 60 trees which appeared potentially 90 to 100 percent compatible in the first stage of testing were grafted with 26 new scion clones for the second stage of testing. Six trees were from the high-intensity test, 54 from the low-intensity test. The total number of grafts was 1,404 (fig. 1). Unions were sacrificed in 1973 and processed by the same methods as first-stage graft unions. Average compatibility of each tree was calculated. Results of the second stage of testing indicated that 16 trees were 90 to 100 percent compatible. These trees were then used to produce compatible rootstocks by rooting and pollination.

Figure 2.--First-stage grafting design and compatibility results for one set of 11 Beaver Creek Seed Orchard trees tested by the low-intensity method.

	GRAFTED AS SCIONS Tree No.										
Tree No.	133	134	135	136	137	138	139	140	141	142	143
GRAFTED AS ROOTSTOCKS (NO RECIPROCAL OR AUTOPLASTIC GRAFTS WERE MADE)											
133	0										
134	I[1]	0									
135	I	C	0								
136	I	C[2]	I	0							
137	C	I	I	C	0						
138	C	C	C	C	C	0					
139	I	C	C	I	I	C	0				
140	I	I	C	C	C	C	C	0			
141	I	C	C	C	I	C	C	C	0		
142	I	C	C	I	C	C	C	I	C	0	
143	C	I	I	C	C	C	I	C	I	I	0
Average compatibility	30	60	60	60	60	100	60	70	70	60	50

[1]Incompatible graft unions.
[2]Compatible graft unions.

Rooting studies on potentially highly compatible trees began in 1972. Cuttings were obtained by clipping current year tips from all first- and second-order lateral branches on the lower 2 m of each ortet. Only the current year's twigs were clipped. The clipping treatment on the lower 2 m resembled the shearing commonly done by growers in shaping Douglas-fir Christmas trees for market (fig. 3). From the 16 trees, 212 ramets were rooted and planted in a rootstock clone bank near Monmouth, Oregon at 1.8-x 3.7-m spacing in January 1974 and 1976. Collecting branch tips for rooting from clone bank ramets was not started until 1978 when they were 2 m tall.

Results and Discussion

No apparent difference in average compatibility was found between the 50- and 253-tree populations (66 vs. 64 percent, respectively) (table 1). This similarity in compatibility may permit some comparisons of the high- and low-intensity tests used in the first stage, even though different populations were used.

Figure 3.--A 16-year-old ortet which has been cultivated for 9 years to produce both seed and branch tips for rooted cuttings.

A number of trees potentially 90 to 100 percent compatible were found with both strategies. Low-intensity tests, with 10 scion clones per tree, indicated that 54 of the 253 trees (21 percent) were 90 to 100 percent compatible. High-intensity tests, with 21 scions on each tree, indicated that 6 trees of the 50 (12 percent) were potentially 90 to 100 percent compatible (table 1).

Second-stage testing of trees found potentially 90 to 100 percent compatible in the first stage revealed that many selections from the low-intensity group were poor choices. Only 11 of the 54 low-intensity selections (20 percent) proved to be 90 to 100 percent compatible in second-stage testing. This 20-percent accuracy compares with the 83 percent accuracy in selecting trees from the high intensity group of 21. Apparent inaccuracy of screening the low-intensity group was also indicated by the low average compatibility (69 percent) of the 54 trees tested, whereas the high intensity selections averaged 88 percent compatibility in second-stage testing (table 1).

Efficiency of the high and low intensity screening strategies was almost identical: 241 and 243 grafts, respectively, were required to discover a single 90-to 100-percent-compatible tree. The half-diallel grafting design used in the low-intensity test reduced the number of required scion/rootstock combinations by 50 percent (from 2,530 to 1,265), i.e., $n(n-1)/2$ combinations for half diallel vs. $n(n-1)$ for the factorial. If the high-intensity test had used the same technique of grafting (using the same trees as either, or both, scions and rootstocks), only 525, not 1,050, first-stage grafts would have been needed and only 136 grafts would be required to identify each 90-to 100-percent-compatible tree.

Table 1--Comparison of high- and low-intensity tests of graft compatibility in Douglas-fir

	Intensity of sample	
FIRST-STAGE TESTING	Low	High
Number of trees tested	253	50
Number of scion clones grafted/tree	10	21
Total grafts made	1,265	1,050
Average percent compatibility of all first-stage trees	64	66
Number trees potentially 90 to 100+ percent compatible	54	06
Percent trees found potentially 90 to 100+ percent compatible	21	12
SECOND-STAGE TESTING		
Number of trees potentially 90 to 100+ percent compatible in stage one	54	06
Number scion clones grafted/tree	26	26
Total grafts made	1,404	156
Average percent compatibility of all trees tested	69	88
Number 90- to 100-percent-compatible trees identified	11	05
Percent trees found 90 to 100 percent compatible	20	83
Percent first-stage trees found 90 to 100 percent compatible	04	10
Average percent compatibility of trees 90 to 100+ percent compatible	97	95
Range in compatibility (percent)	92-100	91-98
Total number grafts needed to correctly identify one 90- to 100-percent-compatible tree	243	241

The results suggest that most Douglas-fir populations contain a number of highly graft-compatible trees. The high intensity first-stage sampling indicated that 10 percent of the population was highly compatible, while the low intensity sampling indicated only 4 percent (table 1). The 10 percent is apparently more accurate since some highly compatible trees were not detected by low intensity tests, due to insufficient sampling, and were therefore excluded from further testing. An organization which, for safety reasons, would like to have at least 10 different rootstock clones in its orchards should test at least 100 trees by the intensive method or 250 trees by the less intensive method described in this study. A better technique would be to combine the accuracy of the high intensity method with the efficiency of the low intensity half-diallel grafting design. A test with the population broken into 26-tree half-diallel sets would capture the benefits of both screening methods.

The 16 ortets selected as 90 to 100 percent compatible after second-stage testing have been cultivated to produce both seeds and branch tips for propagation. The first seed from these was produced in 1977 by two 13-year-old ortets. Significant

numbers of male buds were lacking at that time. By 1980, the 16- to 19-year-old ortets had grown to an approximate height of 10 m. Seven trees had abundant female buds, but male buds were still sparse. Abundant seed production in the future is indicated by the trees' large crowns (fig. 3).

Seed orchard managers have eagerly sought cuttings of the 16 trees to use as rootstocks for their clonal orchards. Approximately 120,000 cuttings have been gathered during the last 7 years from the 16 ortets and 212 rooted ramets of the same clones (table 2). Cuttings were not taken until ramets were at least 2 m tall. Rooting success averaged 50 percent or higher between 1976-79. Three or 4 years of growth after field planting was required for ramets to produce abundant cuttings without adversely affecting the following year's crop. The 212 ramets growing in the rootstock-clone bank will probably produce 50,000 to 100,000 cuttings per year within the next 5 years.

Production of cuttings from the ortets shows signs of decreasing. Progressively smaller and weaker cuttings were obtained in 1978, 1979, and 1980, as more vigorous branch growth has been directed into the upper, unclipped areas of the crown. It might be possible to reverse this trend by not gathering cuttings for a year or two and by applying fertilizer to stimulate vegetative growth. Removing a major portion of the upper crown would also stimulate growth in the lower crown, but such treatment would be detrimental to seed production.

Table 2--Approximate number of cuttings gathered from highly compatible ortets and rooted ramets

Year of collection	No. of cuttings[1]		Total
	From 16 ortets	From 212 ramets	
1974	2,000	0	2,000
1975	4,900	0	4,900
1976	6,200	0	6,200
1977	6,500	0	6,500
1978	10,000	20,200	30,200
1979	10,200	21,000	31,200
1980	10,000	29,000	39,000
Totals	49,800	70,200	120,000

[1]Rounded to the nearest 100 cuttings.

These 16 highly compatible rootstocks are now widely used in clonal seed orchards. Tree improvement organizations on the West Coast root thousands of cuttings each year from them. In addition, control-pollinated seedlings from the same trees have been planted from northern California to northern Washington for evaluation of graft compatibilty. The supply of rootstocks for present and future needs will soon be adequate. New research on compatible rootstocks will focus on continued improvement in overall compatiblity of clonal and seedling lines and on the secondary influence of rootstocks on cone production, tree form, and tree height.

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GPO 992-662